A Mechanism of Color Variation Operating in the West Coast Sea Hare, *Aplysia californica* Cooper

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The wide variation in the coloration of different specimens of *Aplysia californica* Cooper, which inhabits the algal zones from Monterey Bay on the central California coast to the Gulf of California, has been the source of considerable question as to the specific homogeneity of the species. Cockerell (1901) based a new species name, *A. ritteri*, on an especially brightly colored variety. Garstang (1890) experimented with the European *Aplysia* and showed that there was a definite connection between diet and coloration. He believed that the veliger larvae settled in deep water and then migrated from deep water through the red, brown, and green algal zones, taking in turn the color of each.

MacMunn (1899) and Schreiber (1932) have indicated that the normal skin pigment is basically made up of degradation products of the tetrapyrrole molecule of chlorophyll. These are partly porphyrins, which are cyclic tetrapyrrole compounds, and bilins, which are linear chains of pyrrole molecules resulting from a break in the cyclic tetrapyrrole ring. The color and consequent absorption spectrum of these products varies as their structure is changed. Thus bilins from degraded green chlorophyll can produce a variety of blues, browns, greens, yellows, and reds which are all characteristic colors of the bilin compounds.

Many marine algae possess a high concentration of nonchlorophyllic pigments. Among these are the plant bilins or phycobilins, such as red phycoerythrin and the blue-green phycocyanin.

The colors most often seen in the skin of *A. californica* are brown, green, red, and purple, all of which are characteristic bilin colors. The purple pigments are concentrated in the branchial region and are seldom if ever seen elsewhere externally. The basic external body colors are brown, dark green, and grey, with various gradations between them. The red coloration is usually observed in larger specimens from deeper water and is the color most readily proved to be a result of food consumption.

In an effort to determine the relation of color to food, three approaches to the problem were used: (1) the comparison of color pattern and food, as shown by fecal pellet analysis; (2) controlled feeding experiments; and (3) observations on the absorption spectrum characteristics of the pigments.

MATERIALS AND METHODS

Juvenile specimens of *Aplysia californica*, ranging from 4 to 5 inches in length, were captured in the central shore area of Lunada Bay, Palos Verdes, California, and placed in individual pint fruit jars. As soon as a specimen passed a fecal pellet the pellet was collected in a vial and the animal was released in an area far enough removed so as not to be recollected. In this manner a total of 25 individuals was sampled. Pellets were also taken

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from large adult specimens from deeper water in Lunada Bay and off Palos Verdes Point, ¼ mile to the north. The pellets were collected in small, screen-capped vials, and were kept in refrigeration until they could be examined and tabulated.

Numerous specimens of *Aplysia californica* of various color combinations were kept from one to three months in 5-gallon pyrex cylinders supplied with aeration and glass wool–charcoal filters. The sea water was changed as pollution made necessary. These animals were fed on special diets to determine associated color relationships.

Hemolymph was drawn from the hemocoel after the animals had been cooled to refrigerator temperature to render them inactive. This prevented the release of the contaminating purple ink. The blood was centrifuged to remove the cellular elements, and the spectra were run on a Beckman model DU spectrophotometer. Extracts of the pigment of *Plocamium pacificum* were made by grinding the seaweed in a mortar with centrifuged sea hare hemolymph to duplicate the ionic conditions of the purple hemolymph occurring in certain specimens.

Extractions of certain dermal pigments were made with weak hydrochloric acid. Usually 10 per cent v/v was used, although one drop of concentrated hydrochloric acid in 10 cc. of distilled water would dissolve the pigment. The areas of the animal surface from which it was desired to extract pigment were cut off with scissors and placed in the dissolving solutions. These were then studied spectrophotometrically.

EXPERIMENTAL DATA

Fecal Pellets Analysis

The diet of the young specimens of dark green and grey coloration consisted of a wide variety of algae, but the dominant representatives were *Ceramium eitonianum* and *Gigartina canaliculata*. In no case was *Plocamium pacificum* present in noticeable quantity. All of the older specimens had red body streaks and a characteristic purple foot. In every case a varying but very noticeably dominant representation of *Plocamium pacificum* was found to be present. Other greener seaweeds were also present in small quantities.

Controlled Feeding Experiments

Specimens of *Aplysia californica* in all of the available color patterns were kept in the aquaria from one to three months and fed on a diet of parsley leaves and celery tops. These specimens all acquired a uniform pigmentation characterized by a light-brown base color and small dark-brown spots. One large feeding of *Plocamium pacificum* fed to these specimens produced a decidedly pink cast to the base color. This, however, was only temporary.

Two small red animals (2–3 inch) from the *Plocamium* beds north of Palos Verdes Point, apparently similar to the animals mentioned by Berry (1907), were kept in tanks in the laboratory. When captured, these young sea hares were a uniform light pink without markings, although one had a very few pinpoint dots of brown. These animals thus bore no color resemblance to the greenish-brown or red-streaked *Aplysia*. It appeared at first that they constituted a new species, but after two weeks of feeding on parsley leaves they developed the coloration characteristic of *A. californica*. These two animals, fed on parsley leaves, grew to be 4 to 5 inches in length and so closely resembled other animals of similar size and experimental diet but of different initial coloration that it became impossible to differentiate them.

Observations on the Pigments

The blood, or hemolymph, of *Aplysia* is normally a clear fluid without noticeable coloration. However, it was found that specimens feeding principally on *Plocamium pacificum* possessed blood with a strong purple coloration. Spectrophotometric curves were run on the blood and compared with extracts of *Plocamium;* however, while the curves themselves proved interesting, they did not show common absorption maxima with those found in extracts of *Plocamium pacificum* nor did they show a relationship with *Aplysia* purple.

It was also noted that several hours after removing a dead *A. californica* from sea water, the brown epidermal layer of the skin can be rubbed off. In the red-streaked, *Plocamium*-feeding animals it was found that beneath this layer, on the surface of the unpigmented dermal layer, were broad, purplish red streaks of deposited material. In life these streak marks appear red, as the result of the filtering action of the thin epidermal layer. These streaks interconnect to form a reticulum which is most pronounced on the sides, especially near the margin of the foot.

The streaks were rapidly dissolved in dilute hydrochloric acid, dilute acetic acid, and 70 per cent alcohol, the latter changing the gross color to yellow. The acid extract consistently gave a strong absorption peak at 548 mμ (Fig. 1C) with an irregularity in the curve at 490 mμ. The nature of this irregularity suggested to the writer that the curve might be a composite. The streaks were differentially extracted with distilled water and dilute hydrochloric acid. In the former the streak-coated tissue was soaked overnight. The resultant extract was a light yellow and the gross color of the streaks had changed somewhat. The streaks were then completely dissolved in dilute hydrochloric acid in a few minutes.

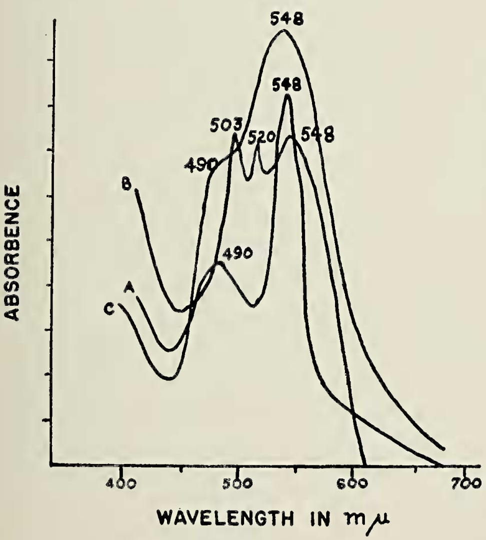

FIG. 1. The curve shown in A is produced by the water soluble fraction of the subcutaneous purple streaks, while that in B is produced by a subsequent acid extraction. The total spectrum of the two components is shown in C. No attempt was made to make the several extracts quantitative.

The water extract showed peaks at 548 and 490 mμ (Fig. 1B). The acid solvent contained a component with absorption maxima at 503, 520, and 548 mμ, the one at 548 being broad, the other sharp (Fig. 1A). No attempt was made to employ quantitative techniques so the relative height of curves A and B are probably not correctly portrayed. When the two are dissolved together the additive effect produced the curve in Figure 1C.

Upon neutralizing the composite extract of the streaks, the color completely disappeared. Upon reacidification the color was reconstituted without significant spectral change, but if made strongly alkaline, a jell-like precipitate formed.

DISCUSSION AND CONCLUSIONS

The reversion of specimens of all available color patterns to a common pigmentation when fed a common diet demonstrated the dependence of these animals on food pigments for their coloration. The land plants which supplied principally chlorophyll as a pigment lacked the special pigments which are normally obtained by these animals from their algal diet. It seemed logical to search for the cause of the red streaks from among these special algal pigments.

The consistence with which *Plocamium pacificum* was found in the fecal pellets of the red-streaked animals led to experiments with that algal form. Feeding experiments involving *Plocamium* over the extended period necessary to produce red streaks were impractical because of the perishable nature of the sea weed. However, single large feedings produced a pinkish cast on the bodies of parsley-fed experimental animals, caused by the blood's becoming temporarily purple.

The lack of common absorption maxima between the extracts of *Plocamium pacificum* and the purple-colored blood is doubtless the result of digestive changes. The significant fact, however, remains that animals feeding on the pinkish purple, phycobilin-pigmented *Plocamium pacificum* develop purple hemolymph. The fact that these purple-blooded specimens also lay down a subcutaneous purple reticulum is very significant, showing the manner in which this particular pigment is transferred from plant to animal pigmentation. It has been demonstrated that the portion of the streaks insoluble in water was soluble in hydrochloric acid. This solubility characteristic suggests the presence of a nitrogen base, perhaps an amine.

SUMMARY

1. Fecal pellet analysis indicated that *Plocamium pacificum* is the major dietary component of red-streaked *A. californica*.

2. The coloration of the widely different color variants could be changed to a homogeneous, indistinguishable pattern by feeding them a diet of parsley and celery leaves.

3. The absorbed pigment from the digested *Plocamium* colors the blood of the sea hare purple.

4. A pigmented compound may then be deposited in a subcutaneous, purple reticulum, having the gross effect of red streaks due to the filtering action of the brown cutaneous layer.

5. Though all the pigments in the chain are of a red to purple color, they have been so changed in the body of the sea hare as not to have common absorption maxima. Their spectra show no affinity with *Aplysia* purple.

6. The streak deposit is composed of not less than two component pigments.

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